A trait-based experimental approach to understand the mechanisms underlying biodiversity–ecosystem functioning relationships

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Running title: A trait-based biodiversity experiment

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Abstract

Plant functional characteristics may drive plant species richness effects on ecosystem processes. Consequently, the focus of biodiversity–ecosystem functioning (BEF) experiments has expanded from the manipulation of plant species richness to manipulating functional trait composition. Involving ecophysiological plant traits in the experimental design might allow for a better understanding of how species loss alters ecosystem processes. Here we provide the theoretical background, design and first results of the 'Trait-Based Biodiversity Experiment' (TBE), established in 2010 that directly manipulates the trait composition of experimental plant communities.

Analysis of six plant traits related to resource acquisition and use were analyzed using principal component analysis of 60 grassland species. The resulting two main axes describe gradients in functional similarity, and were used as the basis for designing plant communities with different functional and species diversity levels. Using such an approach allowed us to manipulate different levels of complementarity in spatial and temporal plant resource acquisition. In contrast to previous biodiversity experiments, the TBE is designed according to more realistic scenarios of non-random species loss along orthogonal axes of species trait dissimilarities. This allows us to tease apart the relative importance of selection and complementarity effects on multiple ecosystem processes, and to mechanistically study the consequences of plant community simplification.

Zusammenfassung

Es wird angenommen, dass funktionelle Pflanzenmerkmale verantwortlich sind für beobachtete Effekte von Pflanzendiversität auf Ökosystemfunktionen, wodurch sich der Fokus der Biodiversitätsforschung von der Betrachtung der Effekte von Pflanzenartenzahlen hin zu der Betrachtung von Pflanzenmerkmalen in einer Gemeinschaft erweitert hat. Die Berücksichtigung von funktionellen Pflanzenmerkmalen hilft möglichweise zu verstehen, wie

der Verlust von Arten Ökosystemprozesse beeinflusst. Unseres Wissens wurde jedoch die funktionelle Ähnlichkeit verschiedener Pflanzenarten zueinander noch nie als Grundlage für ein Grasland-Biodiversitätsexperiment genutzt.

Wir präsentieren den theoretischen Hintergrund, das experimentelle Design und erste Ergebnisse eines sogenannten ‚Trait-Based Biodiversity Experiments' (TBE), welches 2010 im Rahmen des Jena Experimentes etabliert wurde und bei welchem direkt die funktionelle Diversität von Pflanzeneigenschaften manipuliert wurde.

Mithilfe einer Hauptkomponentenanalyse wurden sechs Pflanzenmerkmale von 60 Graslandarten analysiert, die bedeutend für Ressourcenaufnahme und -nutzung sind. Dabei bildeten die Pflanzenarten, die entlang zweier unabhängiger Achsen angeordnet waren, Gradienten in ihrer funktionellen Ähnlichkeit, die als Basis für das Design des TBE dienten.

Auf neu angelegten Versuchsflächen etablierten wir Pflanzengemeinschaften mit unterschiedlicher Pflanzenartenzahl, die sich in ihrer räumlichen und zeitlichen funktionellen Komplementarität unterscheiden. Das neuartige Design des TBE erlaubt es uns in Zukunft den relativen Einfluss von Selektions- und Komplementaritätseffekten auf Ökosystemprozesse zu bestimmen und ermöglicht die mechanistische Erforschung der Konsequenzen von vereinfachten Lebensgemeinschaften.

Keywords: functional diversity, species richness, plant traits, Jena Experiment, selection effect, complementarity effect, redundancy, plant shoot biomass

Introduction

In the past decades an increasing number of studies has experimentally investigated the relationship between plant diversity and ecosystem functions such as plant productivity, carbon and nutrient cycling, and trophic interactions (Cardinale, Matulich, Hooper, Byrnes, Duffy, et al., 2011). Such biodiversity experiments assembled artificial plant communities with different levels of diversity to decouple the manipulated aspects (e.g., plant species richness) from environmental factors (e.g., site fertility), and to test for a causal relationship between biodiversity and ecosystem processes (Schmid & Hector, 2004). With the increasing recognition that plant species richness has a general positive effect on many ecosystem processes (Cardinale, Duffy, Gonzalez, Hooper, Perrings, et al., 2012; Allan, Weisser, Fischer, Schulze, Weigelt, et al., 2013), new questions have arisen about the mechanisms underlying these relationships.

The first generation of biodiversity experiments manipulated plant productivity levels rather than plant species richness (Tilman, Wedin, & Knops, 1996; Huston, 1997), making it difficult to identify the drivers that could explain the observed positive relationship between species richness and productivity (Tilman et al., 1996; Huston, 1997). Subsequent experiments manipulated plant species richness directly and also found positive diversity–productivity relationships (Tilman, Lehman, & Thomson, 1997; Hector, Schmid, Beierkuhnlein, Caldeira, Diemer, et al., 1999). A controversy arose whether these were caused through a selection effect, i.e. the increased probability of selecting a productive species in diverse mixtures when species are added in a fully randomized way, or a complementary effect, i.e. niche complementary (higher resource use efficiency in diverse communities or different pathogen niches) (Loreau et al., 2001). Later experiments directly tested for complementarity effects, e.g. by using large species pools and controlling species richness and functional group numbers (Roscher, Schumacher, Baade, Wilcke, Gleixner, et al., 2004). Criticism on the artificially created plant communities of sown biodiversity

experiments (Leps, 2004) led to alternative experimental approaches such as species removal experiments (e.g. Symstad & Tilman, 2001; Diaz, Symstad, Chapin, Wardle, & Huenneke, 2003; Urcelay, Diaz, Gurvich, Chapin, Cuevas, et al., 2009) with their own limitations of confounding species richness and community density.

Most recently, the focus of biodiversity research has shifted towards manipulating functional (or phylogenetic) diversity directly (Cadotte, Albert, & Walker, 2013), because functional differences among plant species are assumed to be key in understanding diversity–ecosystem functioning relationships. Previous research has shown that the relationship between species richness and functional diversity is complex and context dependent (Cadotte, Carscadden, & Mirotchnick, 2011), and is likely to underlie both selection and complementarity effects (e.g. Tilman, 1999). In addition, it is increasingly accepted that functionally important aspects of biodiversity are better represented through measures of functional trait composition (Díaz & Cabido, 2001) than through traditional functional group assignments (e.g. legumes, forbs, grasses). In this context, functional traits are defined as morphological, physiological or phenological characteristics of an organism affecting its individual performance (Violle, Navas, Vile, Kazakou, Fortunel, et al., 2007). Explicitly manipulating functional trait composition of a plant community is expected to allow a more mechanistic understanding of how species loss alters ecosystem processes (Hillebrand & Matthiessen, 2009). Specifically, functional trait diversity may maximize a given ecosystem function only if species show complementarity along the functional axis of traits most relevant to that function. For example, primary productivity, the variable investigated most frequently in biodiversity experiments, is expected to depend on the degree of functional complementarity in traits related to resource acquisition and use, but ‘functional identity effects’ have also been proposed to play a crucial role in determining ecosystem level properties. Indeed, many recent studies (Díaz, Lavorel, de Bello, Quétier, Grigulis, et al., 2007; Mokany, Ash, & Roxburgh, 2008; Schumacher & Roscher, 2009; Roscher, Schumacher, Gubsch, Lipowsky, Weigelt, et

al., 2012) have shown that both positive selection and complementarity effects explain higher aboveground productivity of grassland communities. If the most productive species are also functionally unique, both the selection and complementarity effects could interact in a given plant community. For instance, Roscher, Scherer-Lorenzen, Schumacher, Temperton, Buchmann, et al., (2011) recently showed that successful mixture species (i.e. species obtaining a larger biomass production in mixtures than expected from their monoculture biomass production) are functionally more different than the average functional distances among species in a given mixture.

Within the framework of the Jena Experiment, we established in 2010 the so-called 'Trait-Based Biodiversity Experiment' (TBE), which directly manipulates plant species composition based on the relative position of plant species along functional axes of trait dissimilarity. The aim was to construct a biodiversity experiment using different levels of complementarity in resource acquisition (spatial and temporal). In contrast to previous biodiversity experiments, the TBE design is based on non-random species loss scenarios along orthogonal axes of trait dissimilarities. Here we provide the theoretical background, methodology and design of the TBE to stimulate further discussion about the design of a 'new generation' of biodiversity experiments and to generate hypotheses and predictions for future studies. We test our experimental design by applying Rao`s Q (Rao, 1982), one of the most accepted indices to quantify functional diversity in experimental communities of ideally similar or the same species richness (see, e,g., Wacker et al., 2009). We illustrate the potential of the TBE by showing first results on how aboveground productivity relates to different levels of plant functional diversity.

Materials and methods

Selection and ordination of plant species traits

For all plant species belonging to the species pool of the Jena Experiment (N = 60 species) (Roscher et al., 2004) we selected six plant traits: (1) plant height, (2) leaf area, (3) rooting depth, (4) root length density, (5) time of growth, and (6) flowering onset. All these traits directly relate to resource acquisition in space (trait 1–4) and time (trait 5–6) to form a diversity gradient in resource-use characteristics. Detailed information about the supposed ecological function of these traits is given in Table 1. Missing values occurred in three of the six traits, but were evenly distributed across species assigned to different functional groups (grasses, legumes, small and tall herbs), comprising eight species for flowering onset and 17 species for root traits. Furthermore, we restricted the TBE to grasses and non-legume herb species, because the important role of legumes in grasslands is already well understood (Mulder, Jumpponen, Hogberg, & Huss-Danell, 2002; Temperton, Mwangi, Scherer-Lorenzen, Schmid, & Buchmann, 2007).

The six traits were analyzed by a principal component analysis (PCA) followed by a 'varimax' orthogonal rotation. The first two principal components explained 66% of the variation among species. The first axis separates species according to their resource use along a vertical spatial gradient (light, water, nutrient). The second axis separates the species' resource use along a temporal gradient (life history, phenology) (Table 1; Fig. 1).

We defined three different species pools of eight species each based on the results of the PCA. Pool 1 covered the entire PCA axis 1 and pool 2 covered the entire PCA axis 2, both at intermediate values of the other axis, whereas pool 3 comprised the extreme species on the two PCA axes combined thus maximizing both spatial and temporal functional diversity (Fig. 1). In the selection of the eight species for each of the pools, a number of criteria had to be fulfilled, based on the knowledge gained from the first phase of the Jena Experiment (Roscher

et al., 2004; Heisse, Roscher, Schumacher, & Schulze, 2007): i) species that establish poorly in monocultures were excluded; ii) each of the three species pools (pool 1, 2 and 3) had to cover the full functional gradient (Fig. 1); iii) if possible, the number of herbs and grasses in each species pool should be balanced. Each species pool was separated into four sectors (Fig. 2) to guarantee a balanced representation of species along the two functional gradients, and two species were selected from each of the four sectors. The final selection of species in the four sectors of each pool was realized by applying the four selection criteria followed by a stratified random sampling (Table 2).

Pool 1 and 2 thus maximize diversity along one functional dimension, defined according to the two leading PCA axes. In contrast, species of pool 3 are located in the corners of the two-dimensional functional trait space described by the two leading PCA axes (Fig. 1), allowing maximization of functional diversity in two dimensions. The range and distance of sectors which are covered by the species in mixtures define the level of functional diversity, which is manipulated in our experiment (see design description below).

On 138 plots of 3.5 x 3.5 m size we manipulated sown plant species richness (PSR; 1, 2, 3, 4 and 8 species) and plant functional diversity (FD_{Jena}; 1, 2, 3 and 4) within each species pool so as to obtain a **complementarity** and a **redundancy part.** In the **complementarity part** we maximized the coverage of the trait space along a functional gradient so that mixtures were composed of species from different sectors (Fig. 3). For instance, a two-species mixtures of the complementarity part of the experiment can vary between FD_{Jena}= 2, 3 and 4, as the two species can come from neighboring sectors (FD_{Jena}= 2), or from the most distant sectors (FD_{Jena}= 4). The **redundancy part** of the experiment comprises plant species communities, where at least two species belong to the same sector (Fig. 3). We additionally created one eight-species mixture per species pool by growing the two species from all four sectors

together (FD_{Jena}= 4). Theoretically, there were more potential redundancy combinations than replicates in our design, and therefore we randomly sampled species within the different combinations of species richness x FD_{Jena}. The resulting PSR x FD_{Jena} combinations and replicates are shown in detail in Fig. 3 and Table 3.

Plant communities were sown in 2009. For details regarding the establishment process, see Appendix A.

First plant community measurements

Plant aboveground biomass was harvested in May and August 2012 by clipping the vegetation 3 cm above ground in two randomly placed rectangles of 0.2 x 0.5 m per plot. Samples were separated into sown species and weeds, dried at 70 °C for 72 h and weighed. The two replicates per plot and sampling campaign were averaged, and data from May and August were summed to get annual aboveground biomass production of target species per plot.

Statistical analyses

To test how the actual (realized) functional diversity of the sown plant communities correlated with the FD_{Jena} levels given by the experimental design we used the functional diversity index Rao's Q (Botta-Dukat, 2005). Sown species proportions were used for the species abundance matrix of each experimental community. To analyze if the species pools differed in functional diversity along the trait axes for which they were assumed to be representative (FD_{Jena} in traits associated with spatial or temporal resource acquisition, and all traits) we calculated Rao's Q values based on different trait sets (Table 1). A first calculation was based on spatial resource acquisition traits (Rao's Q SRT, maximizing the functional gradient for pool 1); a second calculation included temporal resource acquisition traits (Rao's Q TRT, maximizing the functional gradient for pool 2); a third calculation was based on all

six selected plant traits (Rao's Q AT; maximizing the functional gradient for pool 3). All calculations and statistics were carried out with the statistical software R (R development Core Team, http://www.R-project.org. version 2.13.1) including the package 'FD' (Laliberté & Legendre, 2010).

We tested effects of sown plant species richness and plant functional diversity on Rao's Q SRT, Rao's Q TRT and Rao's Q AT with analysis of covariance (function aov using sequential sums of squares) and fitted a model including the design variables block (factor), plant species richness (PSR, numeric), plant functional diversity (FD_{Jena}, numeric), species pool (Pool, factor) and their interactions (PSR x Pool and FD_{Jena} x Pool) as fixed terms. For each species pool we conducted additional linear models to test if correlation strength (adjusted R^2) between Rao's Q and sown plant species richness and plant functional diversity differed between pools. We also calculated CWM (community- weighted mean traits) for each trait and tested for correlations between FD_{Jena} and CWM to evaluate the relationships between designed functional diversity (FD_{Jena}) and functional identity.

In a next step, we tested effects of PSR and FD_{Jena} on annual biomass production (spring plus summer harvest) and per season (spring and summer harvest separately). For each of the response variables we fitted a full model including block, PSR, FD_{Jena}, species pool and their interactions as fixed terms. Full models were simplified using the function `stepAIC´ and non-significant variables were removed from the model. Only the variable `block´ was always retained in the models even if not significant. Aboveground biomass production was square-root transformed to account for non-normality of errors and heteroscedasticity.

Results

Designed functional diversity in the Trait-Based Biodiversity Experiment

Sown plant species richness increased the functional diversity index Rao's Q SRT, Rao's Q TRT, and Rao's Q AT (Table 4, Fig. 4A, C, E). In addition, there was a significantly positive

effect of functional diversity (FD_{Jena}) on Rao's Q, but species pools differed in their correlation strength depending on which plant traits were included into the calculation (Table 4). We found the strongest relationship between FD_{Jena} and Rao's Q SRT in species pool 1 and 3 (Fig. 4B), whereas species pool 2 had the highest correlation between FD_{Jena} and Rao's Q TRT (Fig. 4D). The correlation between Rao's Q AT was strongest in species pool 3 (Fig. 4F). FD_{Jena} and CWM based on single traits did not correlate (see Appendix B).

Plant and functional diversity effects on plant aboveground biomass

Annual biomass production in 2012 was not significantly affected by sown plant species richness, but increased significantly with increasing FD_{Jena} (Table 5). Similarly, early-season biomass production (harvest late May) was unaffected by plant species richness, but slightly increased with increasing FD_{Jena}. In late-season biomass production (harvest late August) the positive effects of FD_{Jena} on productivity disappeared. Annual biomass production as well as its seasonal variation differed strongly between the three species pools, with plant communities belonging to species pool 1 being the most productive and plant communities of species pool 3 being the least productive ones (Table 5, Fig. 5).

Discussion

Novelty and aims of the experiment

Knowledge about functional differences between plant species are necessary to mechanistically understand consequences of biodiversity loss for ecosystem functioning. This idea has been established already early in biodiversity–ecosystem functioning (BEF) research (Tilman, 1999; Loreau, 2000; Petchey & Gaston, 2002). Pre-defined functional groups (such as grasses, herbs and legumes) have been used to create gradients in 'functional diversity' and shown to be drivers of community performance (e.g. Finke & Snyder, 2008; Wacker, Baudois, Eichenberger-Glinz, & Schmid, 2009; Cadotte et al., 2009). The disadvantage of this

rough measure of 'functional diversity' is the lack of consideration of any functional variation among species within functional groups. Therefore, a number of recent studies calculated trait-based functional diversity indices for existing plant communities in biodiversity experiments (Petchey, Hector, & Gaston, 2004; Scherer-Lorenzen, 2008; Viketoft, Bengtsson, Sohlenius, Berg, Petchey, et al., 2009; Wacker et al., 2009; Cadotte et al., 2009; Flynn, Mirotchnick, Jain, Palmer, & Naeem, 2011; Roscher et al., 2012). These studies indeed often show that quantitative measures of trait variation are better predictors for ecosystem processes than species or functional group richness. However, they also indicated that functional identity (quantified as CWM= abundance-weighted community mean traits) is important in explaining ecosystem properties (Roscher et al., 2012). In addition, it has been shown that functional diversity indices based on multiple traits may fail as predictors for ecosystem processes because different traits are associated with different niche axes (Butterfield & Suding, 2013).

Several experiments with microbial communities manipulated phylogenetic or functional diversity alongside species richness (Maherali & Klironomos, 2007; Matias, Combe, Barbera, & Mouquet, 2012), and recently theoretical approaches have proposed to include trait composition in biodiversity experiments (Dias, Berg, de Bello, Van Oosten, Bílá, et al., 2013).

The establishment of the TBE advances functional diversity research by directly manipulating functional diversity instead of a surrogate (phylogeny), considering and separating different functional niche axes (spatial and temporal resource use). In addition to the inclusion of trait-based measures in designing the TBE, the advantages of our particular experimental design are: (1) Effects of plant diversity can be separated better from species identity effects because each plant species is present at each diversity level; (2) The effects of functional diversity can be separated from functional identity (low correlation between FD_{Jena} and CWM); (3) The

response of different ecological functions to functional diversity can be separated from species richness effects; (4) The design is based on plant traits directly measured on the same experimental field site; (5) Plant species span across continuous gradients of trait dissimilarity, allowing for testing the relative importance of complementarity and redundancy. The novel design of the TBE aims to test whether consequences of species loss for certain functions or ecosystem multifunctionality can be predicted from the distribution of species' traits in the community and serves to test a bundle of specific hypotheses, which are in the focus of functional diversity research (e.g. Cadotte et al., 2011), for example:

(1) Increasing the coverage of trait space along a single functional dimension may increase positive diversity effects on ecosystem processes, if trait axes are relevant for the considered process.

(2) Increasing the coverage of trait space in two independent dimensions increases the chance to detect complementarity in several dimensions, in space and in time.

(3) Increasing redundancy within a given level of functional diversity does not enhance ecosystem functions at a given time but across time because species of similar functions can replace each other to prevent functional turnover thus increasing ecosystem stability and multifunctionality.

Designed functional diversity

The comparison of designed (FD_{Jena}) and functional trait diversity (Rao's Q) in the TBE showed that both are highly correlated and that the species pools strongly differ. Increasing FD_{Jena} increased Rao's Q SRT; this linear correlation was strongest in pool 1, which was selected to maximize a functional gradient along the spatial resource use axis. Accordingly, the strongest linear correlation between FD_{Jena} and Rao's Q TRT was found in species pool 2. Rao's Q AT was also highly correlated with FD_{Jena} and this correlation was strongest for pool 3. Sown plant diversity also led to increasing Rao's Q, but irrespective of which traits were

included in the analyses. These results suggest that the design indeed fulfills the hypothesized features, thus enabling to test trait maximization either along two independent functional dimensions (trait axes 1 = pool 1; and trait axis 2 = pool 2) or in a two-dimensional trait space (= pool 3).

Plant and functional diversity effects on plant aboveground biomass

Plant biomass production was not affected by plant species richness, but increased significantly with increasing FD_{Jena}. Furthermore, biomass production strongly differed between species pools, but we found no significant interactions between plant species richness, FD_{Jena} and species pools. However, we here report only short-term results and plant and functional diversity effects may need a longer time to unfold (Reich, Tilman, Isbell, Mueller, Hobbie, et al., 2012; Eisenhauer, Reich, & Scheu, 2012). Although functional diversity effects occurred already early after establishment, we expect them to increase over time (Roscher, Schumacher, Lipowsky, Gubsch, Weigelt, et al., 2013).

The overall high productivity in species pool 1 is probably due to the fact that spatial, in contrast to temporal, resource-use traits are more important for plant productivity (Roscher et al., 2012). The larger importance of spatial resource-use traits may also explain the poor performance of pool 3 in terms of aboveground biomass production. However, it is also possible that the reduced average performance of pool 3 and the high variability among mixtures in this species pool is due to a slow establishment of several species (e.g. *A. sylvestris, S. officinalis, C. oleraceum*; Heisse, Roscher, Schumacher, & Schulze, 2007).

Except for species pool 2, the increase in productivity was highest from FD_{Jena}=1 to FD_{Jena}= 2 and reached maximum values at FD_{Jena}= 3, suggesting complementarity to saturate already at relatively low levels of functional diversity. We further expected to find stronger seasonal differences in communities of pools 2 and 3. Communities of pool 3 differed strongly in their establishment due to the inclusion of very dissimilar species. However, when analyzing

productivity in the different seasons for pool 2 we found higher within-FD level variation at low FD_{Jena} levels (1 and 2) than at high FD_{Jena} levels (3 and 4). This pattern can be explained by the fact that low functional diversity in pool 2 represents communities with high seasonal redundancy leading to either very high or very low productivity at a given sampling date. In contrast, plant communities with high functional diversity have higher seasonal complementary, leading to more stable seasonal productivity levels.

Based on our results we make the following predictions for future results of the TBE based on the regular measurement of several ecosystem processes (e.g. above- and belowground biomass production, microbial biomass) and characterization of trophic interactions (above- and belowground consumers):

(1) We expect a stronger increase in ecosystem processes from low to medium functional diversity (FD_{Jena}) than from medium to high, where a saturation or even decreases in ecosystem processes at high functional diversity levels are possible (Hillebrand et al., 2009).

(2) We expect that maximization of complementarity in spatial resource acquisition traits (here: high FD_{Jena} in species pool 1) leads to increases in all productivity-related measurements. Changes in complementarity of temporal resource acquisition traits (here: FD_{Jena} in species pool 2) should affect measurements which relate to seasonal variation, such as the temporal variability in flower visitation by pollinators. Maximizing trait space in two dimensions (temporal and spatial resource acquisition, here: FD_{Jena} in species pool 3) should lead to the strongest mean effects on multiple ecosystem processes in the long term.

(3) We expect stronger complementarity effects at a given plant richness level for higher functional diversity levels (here FD_{Jena}).

Conclusion

There is a growing consensus that positive effects of biodiversity on ecosystem functioning arise from functional differences among plant species. Current insights into how trait

complementarity and trait redundancy underlie observed biodiversity effects now allow for specific predictions about the performance of particular plant communities. The important next step in functional biodiversity research is thus designing experiments where the trait composition of a plant community is directly manipulated, to directly analyze trait contributions to specific processes and to test the theories derived from modeling and correlative analyses. This is what the trait-based experiment sets out to do. It thus represents a new generation of biodiversity experiments that can serve as a multi-disciplinary platform for studying the mechanisms underlying the effects of biodiversity on ecosystem multifunctionality.

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Appendix A. Supplementary data

Appendix B. Supplementary data

Supplementary data associated with this article can be found in the online version, at xxxxx.

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535 **Table 1.** Description and sampling methods of the selected plant traits. All traits were measured in monocultures, which were established in 2002 in the framework of the Jena-Experiment. Columns 3 and 4 give information about the trait correlations with the two PCA axes (standardized loadings), based on a correlation matrix. Column 5 lists the axis to which the plant trait relates (based on standardized loadings). SRT= spatial resource acquisition trait, TRT= temporal resource acquisition trait.

Plant trait	*Indicator*	*Loadings PC1*	*Loadings PC2*	*Trait category*	*Data collection and detailed information*
Aboveground					
Plant height	direct light interception	0.47	0.27	SRT	Canopy height at estimated peak biomass before mowing was measured in 2004 (Roscher et al., 2011).
Leaf area	direct light interception	0.74	0.43	SRT	Bulk samples of the uppermost fully expanded leaves were collected, pooling material from at least five different plant individuals at estimated peak biomass before first mowing in late May 2004 (LI-3100 Area Meter, LI-COR, Lincoln, USA).
Growth starting date	resource use along a seasonal gradient	0.20	0.85	TRT	Week of the year was based on weekly observations during the growing season in 2004.
Flowering starting date	resource use along a seasonal gradient	0.01	0.90	TRT	Cumulative growing degree days. Day of the year was based on weekly observations during the growing season in 2004. It is well correlated to growth onset (Person's r = 0.65) and, thus, we used this linear relationship to predict the eight missing values for flowering onset.
Belowground					
Rooting depth	deep soil nutrient/ water	0.83	0.13	SRT	Data were obtained by measurements in 2003 using methods described in Bessler, Temperton, Roscher, Buchmann, Schmid, et al. (2009).
Root length density	deep soil nutrient/ water	-0.78	0.24	SRT	Data were obtained by measurements in 2003 using methods described in Bessler et al. (2009).

540

Table 2. Assignment of single species to species pools and sectors (Fig. 2).

Pools	**Sector 1**	**Sector 2**	**Sector 3**	**Sector 4**
Pool 1	*Festuca rubra*	*Avenula pubescens*	*Leucanthemum vulgare*	*Centaurea jacea*
	Poa pratensis	*Phleum pratense*	*Plantago lanceolata*	*Knautia arvensis*
Pool 2	*Holcus lanatus*	*Phleum pratense*	*Dactylis glomerata*	*Anthoxanthum odoratum*
	Geranium pratense	*Plantago lanceolata*	*Leucanthemum vulgare*	*Ranunculus acris*
Pool 3	*Prunella vulgaris*	*Cirsium oleraceum*	*Anthriscus sylvestris*	*Anthoxanthum odoratum*
	Veronica chamaedrys	*Sanguisorba officinalis*	*Rumex acetosa*	*Glechoma hederacea*

Table 3. List of combinations between the design variables PSR and FD_{Jena} and its respective number of replicates per species pool and for the complete design (all three species pools).

Plant species richness (PSR)	**FD_{Jena} 1**	**FD_{Jena} 2**	**FD_{Jena} 3**	**FD_{Jena} 4**	**Replicates/ species pool**	**Total plot number**
1	8	-	-	-	8	**24**
2	4	6	4	2	16	**48**
3	-	4	4	4	12	**36**
4	-	3	4	2	9	**27**
8	-	-	-	1	1	**3**
Replicates/ species pool	12	13	12	9		
Total plot number	**36**	**39**	**36**	**27**		**138**

Table 4. Summary of least-square linear models testing the relationship between TBE design variables and the functional diversity index Rao's Q. Calculations of Rao's Q base on either all selected plant traits or plant traits relevant for spatial or temporal resource acquisition (see Table 1).

	Rao's Q Spatial resource traits		Rao's Q Temporal resource traits		Rao's Q All traits	
	F	**P-value**	**F**	**P-value**	**F**	**P-value**
Plant species richness (PSR)	77.87	< 0.001	47.12	< 0.001	118.74	< 0.001
Functional diversity (FD_{Jena})	34.43	< 0.001	48.65	< 0.001	72.50	< 0.001
Species pool (Pool)	45.50	< 0.001	89.83	< 0.001	106.85	< 0.001
PSR x Pool	8.78	< 0.001	16.70	< 0.001	20.25	< 0.001
FD_{Jena} x Pool	8.23	< 0.001	13.41	< 0.001	13.51	< 0.001

555 **Table 5.** Summary of least-square linear models testing the effects of TBE design variables on plant shoot biomass 2012. Models were simplified stepwise by removing the least significant variables.

	Annual plant biomass [g/m^2/year]		Plant biomass spring [g/m^2]		Plant biomass summer [g/m^2]	
	F	**P-value**	**F**	**P-value**	**F**	**P-value**
Block	1.55	0.215	0.18	0.833	3.76	0.026
Plant species richness (PSR)	-	-	-	-	-	-
Functional diversity (FD_{Jena})	5.74	0.018	4.04	0.046	-	-
Species pool (Pool)	20.41	< 0.001	20.00	< 0.001	4.23	0.016
PSR x Pool	-	-	-	-	-	-
FD_{Jena} x Pool	-	-	-	-	-	-

Figure 1. Ordination of the 48 non-legume species from the species pool of The Jena-Experiment along the first two axes using Principal Component Analysis (separated by grasses, small herbs and tall herbs). The analysis was based on six plant traits relevant for spatial resource acquisition (Pool 1), temporal resource acquisition (Pool 2) and a combination of both (Pool 3). PCA and rotation was done by including all 60 species of the species pool.

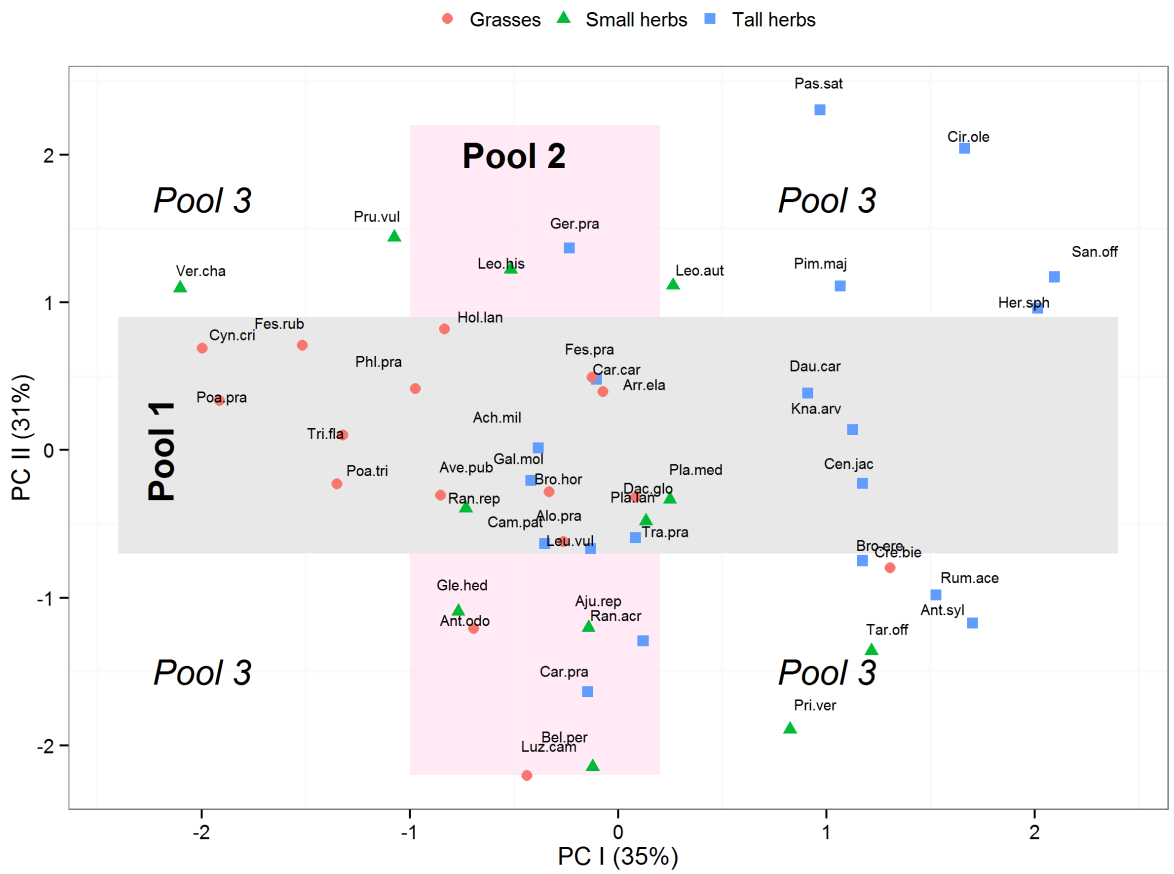
Figure 2. Schematic representation of the allocation of species pools 1, 2, 3 to four different sectors (based on Fig. 1). The range and distance of sectors covered by a plant community define its FD_{Jena}.

Figure 3. Schematic representation of the experimental design. The main figure and the upper insert show the relationship between functional diversity (FD_{Jena}) and plant species richness. FD_{Jena} along a given trait gradient is defined as the range of sectors covered by the species forming plant mixtures. In the species complementarity part, mixtures are composed of plant species that do not belong to the same sectors. In the redundancy part, mixtures comprise at least two species belonging to the same sector.

Figure 4. Functional diversity index (Rao's Q) along a gradient of sown plant species richness (A, C, E) and sown functional diversity (FD_{Jena}) (B, D, F) for the three different species pools. Calculations of Rao's Q are based on the six selected plant traits of the TBE (E and F) and the density of plant species during sowing (proportional seed density). For panels (A) and (B) plant traits relevant for spatial resource acquisition (see Table 1) were taken for the calculation of Rao's Q and in panels (C) and (D) traits regarding temporal resource acquisition were used. Dots show mean values ± standard errors (SE).

Figure 5. Effect of plant functional diversity (FD_{Jena}) on (A) annual plant shoot biomass in 2012, plant shoot biomass in (B) spring and (C) summer for the three different species pools. Dots show mean values ± standard errors (SE).

Figure 1:


Grasses
Small herbs
Tall herbs
Pool 1
Pool 2
Pool 3
Pool 3
Pool 3
Pool 3
Pas.sat
Cir.ole
Pru.vul
Ger.pra
San.off
Ver.cha
Leo.his
Leo.aut
Pim.maj
Her.sph
Hol.lan
Fes.rub
Cyn.cri
Fes.pra
Phl.pra
Car.car
Dau.car
Arr.ela
Poa.pra
Kna.arv
Ach.mil
Tri.fla
Gal.mol
Cen.jac
Poa.tri
Ave.pub
Bro.hor
Pla.med
Dac.glo
Ran.rep
Pla.lan
Alo.pra
Cam.pat
Tra.pra
Lou.vul
Bro.ere
Cre.bie
Rum.ace
Gle.hed
Aju.rep
Ant.syl
Ant.odo
Ran.acr
Tar.off
Car.pra
Pri.ver
Bel.per
Luz.cam
PC II (31%)
PC I (35%)
2
1
0
-1
-2
-2
-1
0
1
2

590

Figure 2:

595

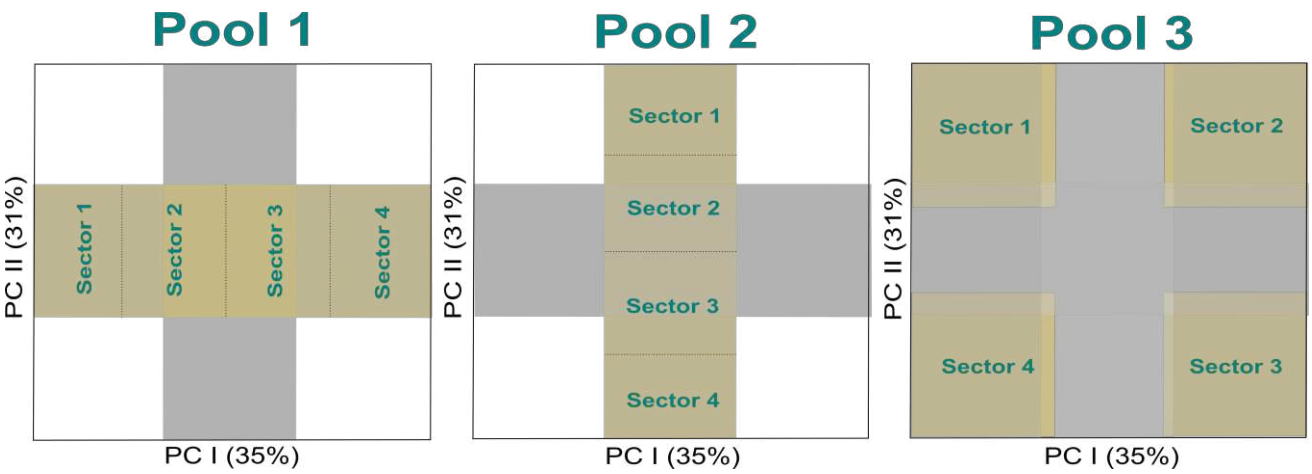
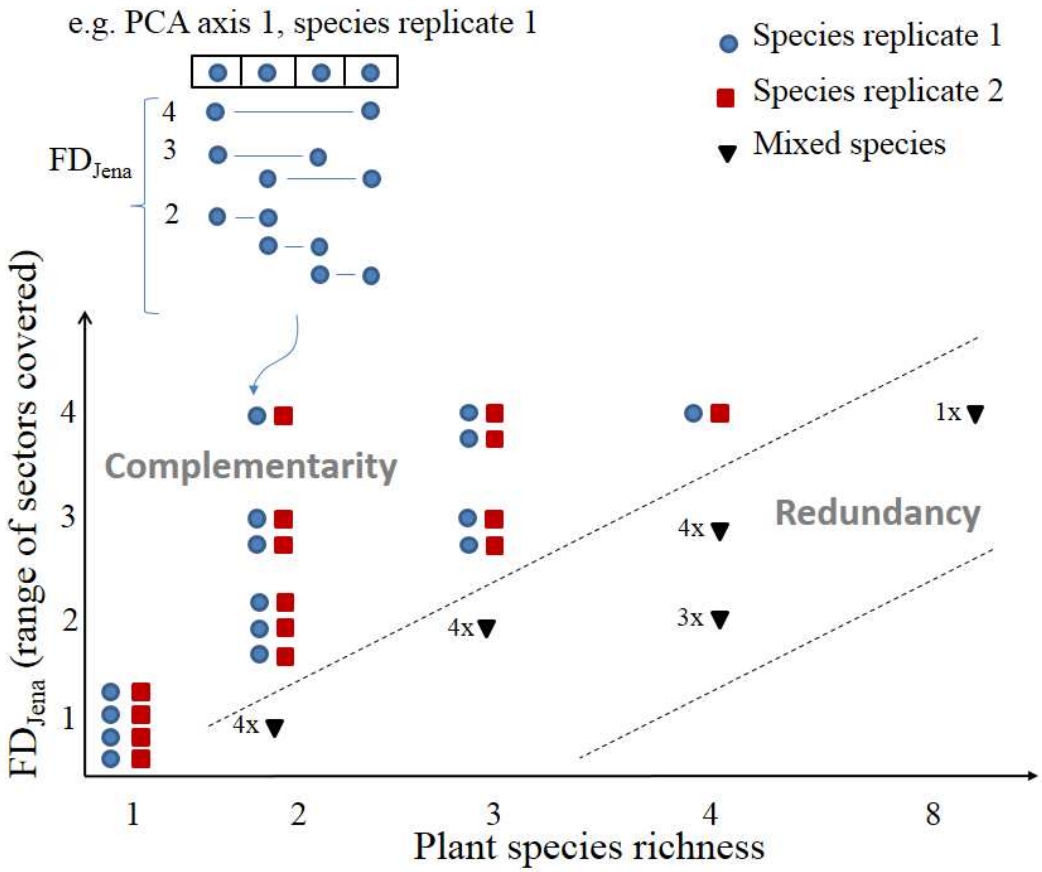

Pool 1
Pool 2
Pool 3
PC II (31%)
PC I (35%)
Sector 1
Sector 2
Sector 3
Sector 4

Figure 3:


e.g. PCA axis 1, species replicate 1
Species replicate 1
Species replicate 2
Mixed species
FD$_{Jena}$
4
3
2
Complementarity
Redundancy
1x
4x
4x
3x
4x
FD$_{Jena}$ (range of sectors covered)
1
2
3
4
1
2
3
4
8
Plant species richness

600

Figure 4:

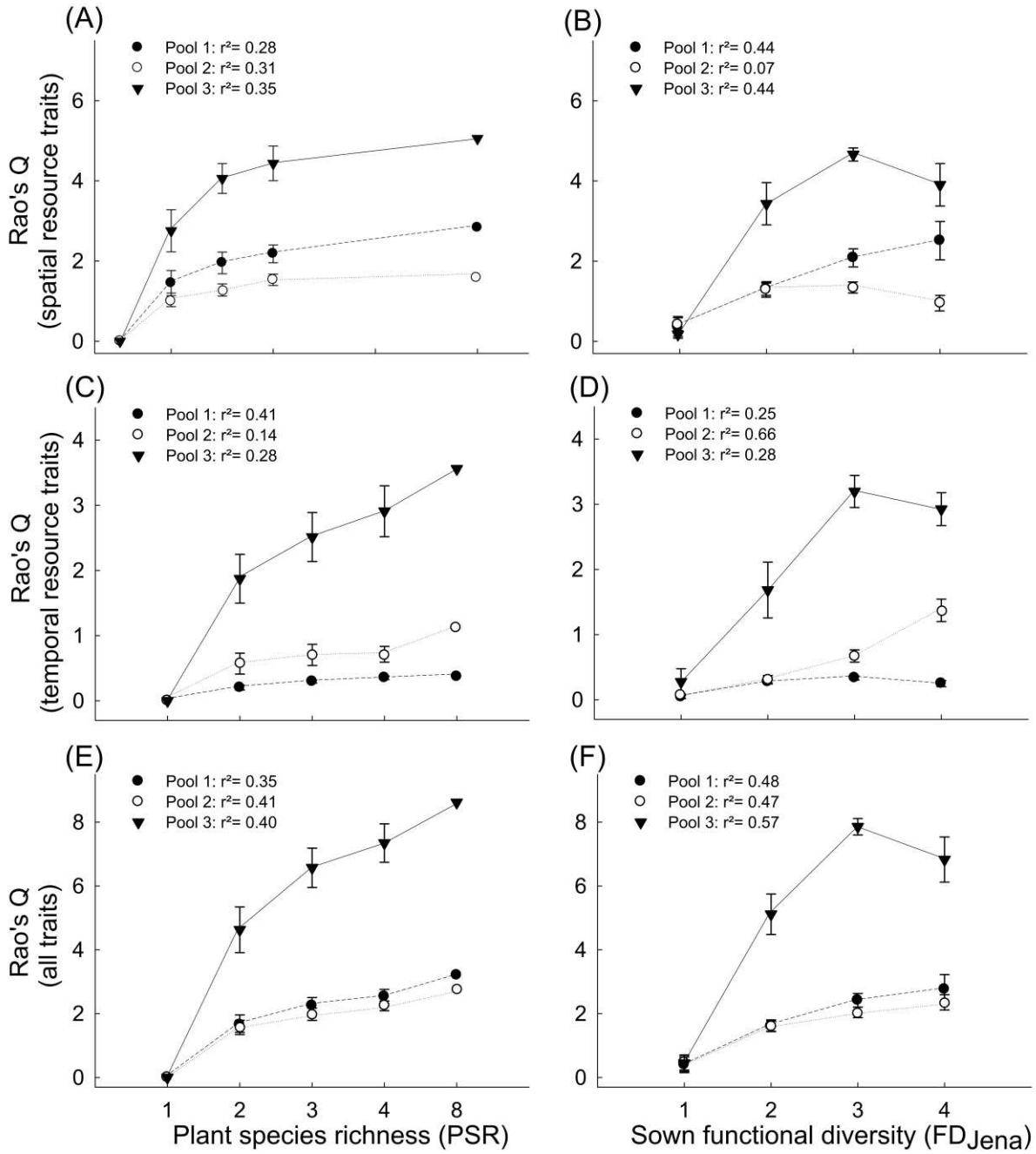
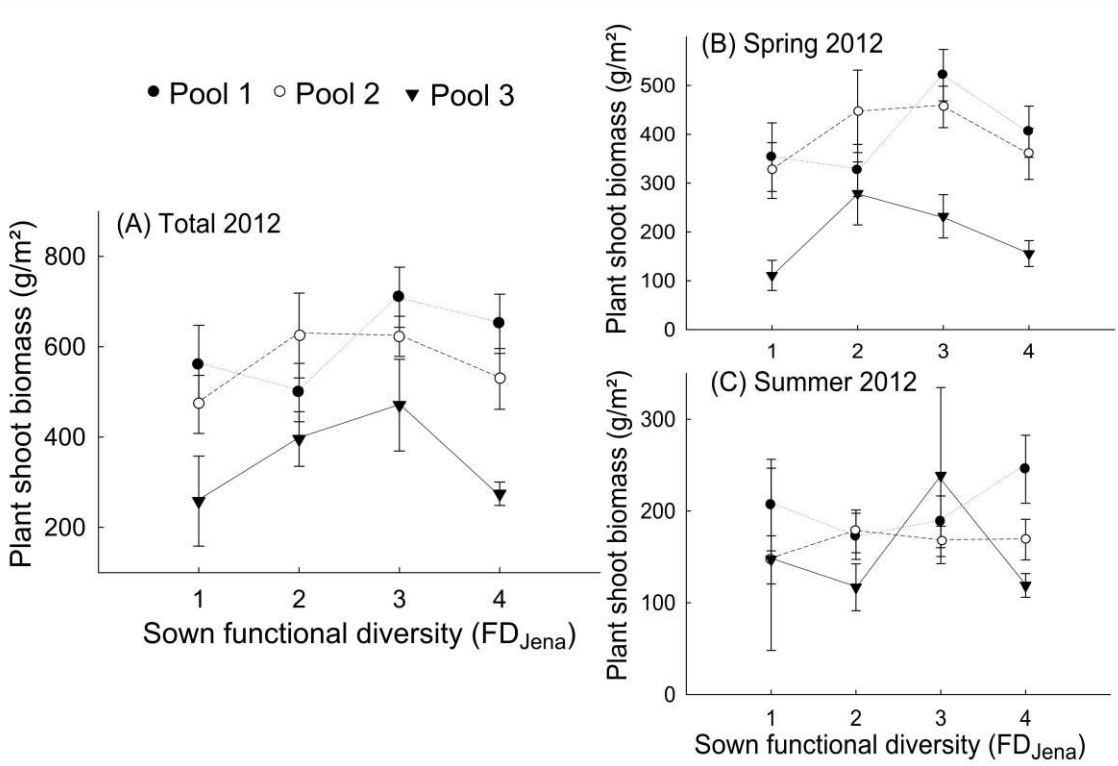

(A)
Pool 1: r²= 0.28
Pool 2: r²= 0.31
Pool 3: r²= 0.35
Rao's Q (spatial resource traits)
(B)
Pool 1: r²= 0.44
Pool 2: r²= 0.07
Pool 3: r²= 0.44
(C)
Pool 1: r²= 0.41
Pool 2: r²= 0.14
Pool 3: r²= 0.28
Rao's Q (temporal resource traits)
(D)
Pool 1: r²= 0.25
Pool 2: r²= 0.66
Pool 3: r²= 0.28
(E)
Pool 1: r²= 0.35
Pool 2: r²= 0.41
Pool 3: r²= 0.40
Rao's Q (all traits)
(F)
Pool 1: r²= 0.48
Pool 2: r²= 0.47
Pool 3: r²= 0.57
Plant species richness (PSR)
Sown functional diversity (FDJena)

Figure 5:


Pool 1
Pool 2
Pool 3
(A) Total 2012
(B) Spring 2012
(C) Summer 2012
Plant shoot biomass (g/m²)
Sown functional diversity (FDJena)

605